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1 ***Actinomyces* species isolated from breast infections**

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30 **ABSTRACT**

31 Actinomycosis is a chronic infection caused by *Actinomyces* species
32 characterized by abscess formation, tissue fibrosis, and draining sinuses. The
33 spectrum of infections caused by *Actinomyces* species ranges from classical
34 invasive Actinomycosis to a less invasive form of superficial skin and soft
35 tissue infection. We present a review detailing all *Actinomyces* species
36 isolated from breast infections in NHS Lothian between 2005 and 2013,
37 *Actinomyces* species isolated from breast infections referred to the UK
38 Anaerobe Reference Unit between 1988 and 2014 and cases describing
39 *Actinomyces* breast infections published in the medical literature since 1994.
40 *Actinomyces* species are fastidious organisms which can be difficult to identify
41 and are likely to be under ascertained as a cause of breast infections. Due to
42 improved diagnostic methods they are increasingly associated with chronic,
43 recurrent breast infections and may play a more significant role in these
44 infections than has previously been appreciated.

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47 **Keywords**

48 Actinomycosis, Actinomyces, diphtheroids, breast infection, breast abscess.

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54 INTRODUCTION

55 Actinomycosis is a chronic, invasive, progressive and often relapsing
56 granulomatous infection caused by Gram-positive, facultatively anaerobic rod
57 shaped bacteria belonging to the genus *Actinomyces*. Classical
58 Actinomycosis in humans is typically caused by *Actinomyces israelii* and is
59 characterized by deep invasive abscess formation, tissue fibrosis and draining
60 sinuses affecting cervicofacial, thoracic, abdominopelvic areas (1). A number
61 of more recently described *Actinomyces* species and Actinomyces-like
62 organisms have been associated with less invasive superficial soft tissue
63 infections and are isolated from abscesses at various anatomical sites (2,3,4).

64 Breast infections are frequently encountered in primary care and breast
65 clinic settings. They can occur in the parenchyma of the breast or the
66 overlying skin and may be in lactating or non-lactating breasts (5). Lactating
67 breast infections are usually caused by *Staphylococcus aureus* (6). The
68 microbial etiology of non-lactating breast infections, particularly those which
69 are chronic or recurrent, is more variable, often polymicrobial and
70 predominantly anaerobic (5,7). A study has demonstrated that when culture
71 methods are used which enhance recovery of fastidious anaerobic organisms,
72 almost 25% of recurrent breast abscesses (8 out of 33 patients) and 10% of
73 primary breast abscesses (2 out of 19 patients) isolated *Actinomyces* species
74 from non-puerperal breast infections (7). However, primary *Actinomyces*
75 infection of the breast, first described by Ammentorp in 1893 (8), is generally
76 considered to be rare. A clinical review from 1994 reported 19 cases
77 described in the English language literature (9). Diagnosis of *Actinomyces*
78 breast infection was often made following surgical intervention although the

79 method of diagnosis was not specified. Duration of symptoms was reported to
80 range from 1-8 years, with two thirds of the patients presenting with recurrent
81 abscesses. The remaining third of the patients were reported to have
82 examination findings suggestive of malignancy. Most of the patients received
83 extensive surgical treatment, with 11 patients undergoing a mastectomy,
84 presumably for management of infection as only 3 were performed on patients
85 with suspected malignancy. Subsequent to this review, case reports and
86 studies have been published describing a further 27 breast infections caused
87 by *Actinomyces* species (table 3).

88 *Actinomyces* breast infection is likely to be under ascertained in routine
89 clinical practice, as these fastidious organisms are notoriously difficult and
90 laborious to identify using conventional laboratory methods (2,3,4).

91 Subsequently there is a risk that cultures of *Actinomyces* species are simply
92 identified morphologically as “diphtheroids” and dismissed as skin
93 commensals, even when grown from an abscess sample. However, new
94 methods of identification such as matrix-assisted laser desorption ionization–
95 time of flight mass spectrometry (MALDI-TOF MS) allow rapid and reliable
96 identification of many bacteria, including *Actinomyces*-like organisms
97 (10,11,12). MALDI-TOF MS and similar technologies are increasingly being
98 adopted by routine diagnostic laboratories worldwide (13).

99 We present a series describing all cases of *Actinomyces* species
100 isolated from breast infections at the Edinburgh Breast Unit over an 8 year
101 period from 2005-2013. Further to this we include data from *Actinomyces*
102 species isolated from breast samples which were referred to the Anaerobe
103 Reference Unit, Cardiff between 1988 and 2013. We then summarize the

104 findings of case reports describing *Actinomyces* species causing breast
105 infection published since the review from 1994 (9).

106

107

108 **MATERIALS AND METHODS**

109 **Bacterial isolates.** Review of electronic records of breast fluid aspirates
110 received between 2005 and 2013 at the microbiology laboratory at the Royal
111 Infirmary of Edinburgh identified eleven cases of breast infections with
112 *Actinomyces* species and one case with the *Actinomyces*-like organism
113 *Actinobaculum schaalii*. Specimens were collected either as pus in sterile
114 containers or on swabs (Stewarts media) and routinely transported to the
115 laboratory. Fastidious anaerobic agar with horse blood (not pre-reduced) was
116 used for culture, incubated in an anaerobic cabinet (80% nitrogen, 10% CO₂,
117 10% hydrogen) for at least 48 hours. Until 2011, Gram-positive rods were
118 identified using biochemical methods, generally API Coryne (bioMérieux).
119 From 2011 onwards isolates were identified using MALDI-TOF MS (Bruker
120 Daltonics).

121 The UK Anaerobe Reference Unit in Cardiff, Wales (UKARU) provided
122 details of *Actinomyces* isolates referred to them from hospitals throughout the
123 UK between 1988 and 2014, where the source stated on the request form
124 was 'breast' (abscess/fluid/wound).

125 **MALDI-TOF mass spectrometry.** MALDI-TOF MS identification was carried
126 out using a Bruker MicroFlex LT mass spectrometer (Bruker Daltonics) and
127 Bruker FlexControl V3.3 software. Isolates were analyzed using a formic acid-
128 based direct, on-plate preparation method. A thin smear of organism was

129 applied to a target plate using a cocktail stick, allowed to dry and then 1µl of
130 100% formic acid was placed on top and allowed to dry. This mixture was
131 overlain with 1µl of matrix solution (cyano-4-hydroxycinnamic acid) and
132 allowed to dry prior to analysis using the MALDI Biotyper. Manufacturer-
133 recommended cutoff scores were used for identification, with scores of >2.000
134 indicating identification to the species level, scores between 1.700 and 1.999
135 indicating identification to the genus level, and scores of <1.700 indicating no
136 identification.

137 **Molecular identification.** Definitive molecular identification was by 16S
138 sequencing using the following method. 16S rDNA was extracted using a
139 chelex resin/boiling method and amplified by PCR using the universal primers
140 pA & pH'. After purification (Qiaquick PCR purification kit #28106, Qiagen), a
141 second PCR reaction was performed using a primer internal to the initially
142 amplified region ('kk') and dye-terminated nucleotides (Big Dye 3.1 Terminator
143 Ready Reaction kit). After a second purification step, the sequence of bases
144 was detected by size / dye terminator of the resulting DNA fragments (ABI,
145 3100). The sequences were compared locally with those of other bacteria
146 (ARU bespoke database, Bionumerics, Applied Maths) or with those listed in
147 international databases (NCBI, BLAST®), with 16S rDNA homology of >97%
148 used to determine bacterial species.

149 **Clinical review.** Paper and electronic patient records of cases were reviewed
150 for information on; age, smoking history, diabetes, nipple piercing, steroid use
151 and whether the patient was lactating at the time of infection. The number of
152 times the patient came into contact with the Edinburgh breast unit was
153 recorded, along with examination findings and management received.

154 Information was collected on GP clinic appointments for breast infections,
155 along with the type, duration and number of antibiotic courses for breast
156 infection in the community.

157 Literary review of published cases was completed on Pubmed and
158 Ovid databases using the keywords: actinomyces, actinomycosis, breast,
159 infection, abscess. Cases of Actinomyces infection of the breast published
160 with clinical descriptions between 1994-2013 are detailed in table 3. Two
161 cases from 1987 not included in the 1994 review (9) are included in this table.

162

163

164 **RESULTS**

165 Table 1 – Cases of *Actinomyces* species isolated from breast infections at the
166 Edinburgh Breast Unit over an 8 year period from 2005-2013.

167

168 Over an 8 year period (2005-2013) we identified eleven cases of breast
169 infections at our center caused by *Actinomyces* species and one case with the
170 Actinomyces-like organism *Actinobaculum schaalii*. The predominant
171 *Actinomyces* species isolated from our subgroup of patients were
172 *Actinomyces europaeus* (n=5), *A. neuui* (n=3) and *A. radingae* (n=3).
173 Identification using MALDI-TOF MS was attempted for 11 isolates and all of
174 these correlated at species level with the definitive molecular identification,
175 with p scores ranging between 1.779 and 2.331. Co-infecting organisms were
176 present in half of these cases (n=6), usually unidentified 'anaerobes'. Ten out
177 of 12 cases (83%) had chronic, recurrent infection ranging from 2-8 (mean
178 2.8) episodes, some over many years. Three patients had hidradenitis

179 suppurativa, 6 patients were smokers and 4 were diabetic with 3 of these
180 patients having a combination of risk factors. No patients in our cohort had a
181 lactational breast abscess and there was no record of any patients having had
182 a nipple piercing.

183 Case number 5 in particular highlights the difficulties associated with
184 diagnosing and managing *Actinomyces* breast infections. This patient had 7
185 episodes of breast infection and abscess formation over a 10 year period
186 treated with short antibiotic courses. Cultures of aspirated abscess material
187 repeatedly failed to grow organisms or were reported to grow “diphtheroids” of
188 uncertain significance. Following a positive growth of *A. radingae* she
189 received a 3 month course of antibiotics and has since had no further
190 relapses (almost 2 years later).

191

192

193 Table 2 – *Actinomyces* species isolated from breast infections
194 referred to the UK Anaerobe Reference Unit from UK hospitals 1988-2014.

195

196 Over a 26 year period (1988-2014), 61 isolates identified as *Actinomyces*
197 species from breast infections were referred to the UK Anaerobe Reference
198 Unit (UKARU) from UK hospitals. Although not considered ‘true anaerobes’,
199 the UKARU has developed extensive expertise over many years regarding
200 *Actinomyces* species. This was driven largely by a referral demand from UK
201 users for advanced identification of clinically relevant isolates initially
202 categorized as ‘anaerobic gram positive rods’ or ‘anaerobic coryneforms’. It is
203 likely that the cases listed here represent only a small proportion of UK cases,

204 as referral of isolates to the unit is not mandatory. Unfortunately a further
205 limitation of the referral process is that clinical information is not available for
206 many of these cases, however a small number (n=5) state either 'recurrent' or
207 'previous breast abscess'. One case worthy of particular mention states
208 'recurrent breast abscess for 11 years' from which *A. radingae* was isolated.

209

210

211 Table 3 – Published cases of *Actinomyces* species isolated from breast
212 infections reported with clinical details since 1994 (ref 14-28).

213

214 Fifteen cases of *Actinomyces* breast infection were identified on literature
215 review between 1994 and 2013, with another 12 cases (7,14) found prior to
216 1994, not included in the Jain et al review (9). This paper therefore reports an
217 additional 27 published cases of *Actinomyces* breast infection to the 19
218 reported in 1994, although clinical details are only available for 17 of these 27
219 cases. There was no clearly predominant *Actinomyces* species. Five cases
220 reported co-infecting anaerobes (17,24) and one case *Staphylococcus aureus*
221 (20), with no mention of co-infecting organisms in 11 of 17 cases.

222 Seven cases were recurrent infections and a range of different treatment
223 combinations were required to reach abscess resolution. This ranged from 2-3
224 weeks of oral antibiotics, to incision and drainage with prolonged antibiotics
225 for 2-6 months, with the most extreme being that of tumorectomy of the breast
226 (22). As with our cohort of patients, prior to the diagnosis of *Actinomyces*
227 breast infection, some patients had been repeatedly treated without success
228 (14,17,20).

229

230

231 Table 4 – Combined number and species of *Actinomyces* breast infections

232 from tables 1-3 and from reference 7.

233 * Two Lothian cases had two different *Actinomyces* sp. isolated

234 † Isolates referred from Lothian are removed from this column to avoid double

235 counting

236 ‡ Published cases include 10 cases from ref 7 with no clinical details

237

238 The *Actinomyces* species most commonly isolated from breast infections

239 according to this combined table (n=102) are *A. neuii* (n=19), *A. europaeus*

240 (n=18), *A. turicensis* (n=16), *A. radingae* (n=15) and *A. odontolyticus* (n=10).

241 These mostly belong to the group of *Actinomyces* species generally

242 considered to be less invasive, although it is noteworthy that in the Lothian

243 and UK cohorts the cases with the greatest number of relapses all isolated *A.*

244 *radingae*. The distribution of *Actinomyces* species broadly reflects previous

245 findings regarding superficial *Actinomyces* soft tissue infections (2,3,4),

246 although these studies did not look specifically at breast infections.

247 Within the NHS Lothian and the published cases 48% (n=14) presented

248 clinically with an abscess, 33% (n=9) presented with a breast mass, 10%

249 (n=3) with a fistula and 7% (n=2) presented with periductal mastitis. There

250 was an average of 2.8 episodes of infection per patient in the NHS Lothian

251 cases. Within the published cases, excluding case 1 who had numerous

252 yearly episodes of recurrent infection for 23 years, there was an average of

253 1.5 episodes of *Actinomyces* breast infection per patient.

254

255

256 **DISCUSSION**

257 Humans and animals are the natural reservoirs of *Actinomyces* species,
258 which until recently have not been found to exist freely in nature (29). Their
259 normal habitat is the mucosal membranes of the oropharynx, gastrointestinal
260 tract and female genital tract. They are inherently low virulent and may rely on
261 the presence of co-pathogens, such as anaerobic bacteria, to enhance
262 pathogenicity (1). Disruption of the mucosal barrier is the usual precursor to
263 infection with *Actinomyces* species and in the breast, the ductal system may
264 serve as a portal of entry. Actinomycosis of the breast usually presents as a
265 chronic, recurrent abscess which in some cases can be difficult to distinguish
266 from inflammatory carcinoma (9,15,26). Fistulas and purulent or bloody
267 discharge from sinuses may occur which may discharge “sulfur” granules
268 (26). In advanced prolonged cases, fibrosis with architectural distortion of the
269 breast tissue is present on mammography (15).

270 The pathogenesis and true pathogenic role of various *Actinomyces*
271 species isolated from breast infections and the treatment required for this has
272 not been clearly defined. This is further complicated by the uncertain etiology
273 of different types of chronic abscess-forming inflammatory conditions involving
274 the breast, from which *Actinomyces*-like organisms can be isolated, such as
275 granulomatous lobular mastitis, hidradenitis suppurativa and periductal
276 mastitis. Granulomatous lobular mastitis presents as a peripheral
277 inflammatory mass which may simulate malignancy or infection. Patients with
278 this condition often develop multiple and recurrent abscesses. It has been

279 suggested that the *Corynebacterium* spp play a part in this condition, (30) but
280 antibiotics effective against these organisms rarely lead to resolution of
281 disease and thus they may not have a major etiological role. Hidradenitis
282 suppurativa is an inflammatory disease of unclear etiology which commonly
283 affects the axilla and groin and can also affect the skin of the lower half of the
284 breast, resulting in recurrent episodes of abscess formation (31). Recent
285 evidence suggests that anaerobic actinomycetes may be involved in the
286 disease process, especially when lesions are more severe (32). Periductal
287 mastitis is a condition linked to cigarette smoking (33) in which the subareolar
288 ducts are damaged and become infected, often by anaerobic bacteria (34).
289 Women may present with subareolar inflammation, abscesses and fistulas
290 (35). Smoking has consistently been identified as a risk factor for primary
291 breast abscess and its recurrence (5,35,36). Other factors, such as diabetes
292 mellitus, obesity, African-American origin and nipple piercing have less
293 consistently been associated with breast abscesses (5,35).

294 Despite finding 12 cases over 8 years at our center, which is
295 comparable to the number of cases described in the medical literature over
296 the same time period, we suspect that there were many missed
297 identifications. During the 8 year study period, we found another 15 cases in
298 Lothian where potential Actinomyces-like organisms were isolated from
299 recurrent breast abscesses, but further identification was not attempted and a
300 report was sent out describing 'diphtheroids' of doubtful or uncertain
301 significance. In addition, 4 out of our 12 culture positive cases had previous
302 samples with isolates of potential Actinomyces-like organisms reported as
303 'diphtheroids' of doubtful significance. This supports the assumption that

304 *Actinomyces* breast infections may easily go undiagnosed in routine clinical
305 practice. *Actinomyces* species are slow to grow and notoriously difficult to
306 identify using conventional laboratory methods, often requiring reference
307 laboratory referral for reliable identification. When *Actinomyces* species do
308 grow on culture they can resemble other diphtheroid-like Gram-positive rods,
309 such as *Corynebacterium* species, many of which are considered to be part of
310 normal skin flora. *Actinomyces* species which are isolated from breast
311 abscess samples may therefore be presumptively identified in the laboratory
312 as 'diphtheroids' based on their morphology and reported as 'diphtheroids' of
313 doubtful or uncertain significance. However, laboratories are increasingly
314 adopting new methods of identification, such as MALDI-TOF MS (13), which
315 allow rapid and increasingly reliable identification of this problematic group of
316 organisms (10,11,12). Indeed, most of the cases in Lothian were identified
317 after 2012, which is shortly after our laboratory started using MALDI-TOF MS.
318 With 10 cases diagnosed in 2 years of using MALDI-TOF MS compared to 2
319 cases over 7 years without MALDI-TOF MS, it is clear that ease of
320 identification is a major factor in the increased recognition of *Actinomyces*
321 breast infections in our clinical setting. The Anaerobe Reference Unit (ARU)
322 has seen a similar increase in the number of isolates referred to them, with
323 more isolates (n=26) referred to them over the last 3 years of the recorded
324 period than had been referred over the first 20 years (n=25). Based on
325 information from referring laboratories, this increase is almost certainly driven
326 by an improvement in the identification of *Actinomyces* species due to
327 increased use of MALDI-TOF MS. Subsequently, UK laboratories unfamiliar

328 with these organisms refer them to the ARU for confirmation of identification,
329 susceptibility testing and clinical advice.

330 Our results support previous findings that *Actinomyces* species can be
331 reliably identified using MALDI-TOF MS (10,11,12), with all 12 of our tested
332 isolates identified to species level, as confirmed by molecular testing. Five
333 isolates were correctly identified to species level by MALDI-TOF MS despite
334 identification scores only reaching genus level confidence ($p < 2.0$). This is in
335 keeping with recent evidence suggesting that the cut-off for species level
336 identification could be reassessed and perhaps lowered to $p \geq 1.7$ for this
337 group of organisms (10,12).

338 We have modified the approach to how organisms from breast samples
339 are identified in Lothian. Breast abscess samples now receive anaerobic
340 incubation for 5 days, along with prolonged *Actinomyces* cultures when
341 clinical details mention chronic or recurring infection. Any Gram-positive
342 bacillus growing from a breast abscess sample is identified using MALDI-TOF
343 MS and should no longer be reported as a “diphtheroid” of uncertain
344 significance without an attempt being made to identify the organism.

345 The primary management of breast abscess is drainage, along with
346 antibiotic therapy appropriate for the underlying cause of the abscess (31).
347 When *Actinomyces* species are isolated, longer courses of antibiotics should
348 be considered. Treatment of classical, invasive Actinomycosis, typically
349 caused by *Actinomyces israelii* and to a lesser extent *A. gerencseriae*, *A.*
350 *meyeri*, *A. odontolyticus* and *A. viscosus/naeslundii* (2,4,37), involves
351 prolonged antibiotic therapy. Textbooks commonly advise 2-6 weeks of
352 intravenous penicillin followed by 6-12 months of oral penicillin or amoxicillin

(38). However, there is evidence that shorter antibiotic courses of under 3 months may be sufficient in some cases (39), particularly those caused by less invasive *Actinomyces* species, such as *A. europaeus*, *A. funkei*, *A. neuii*, *A. radingae* and *A. turicensis* (2,3,4,37,40). Even shorter 7-14 day courses of oral antibiotics are typically used when treating breast infections, but this is likely to be insufficient for *Actinomyces* associated breast infections and longer courses, in addition to surgical drainage, may be required to prevent recurrences. *Actinomyces* species are susceptible to many beta-lactam antibiotics, with penicillin and amoxicillin generally regarded as first choice options (38,41). However, due to the common presence of co-infecting, beta-lactamase producing organisms, treatment options should ideally include beta-lactamase stable antibiotics, such as amoxicillin plus clavulanic acid (41), at least for the initial 2 weeks of treatment. Alternative agents for patients with penicillin allergy could include doxycycline or clindamycin, although there is less evidence for their efficacy (38). We suggest at least 6 weeks of antimicrobial treatment for extensive infections involving *Actinomyces* species or in cases where recurrences have occurred. Although some recurrent cases of *Actinomyces* breast infections seem to have benefited from this, it is not clear whether a longer course of antimicrobial in the first instance would have prevented relapses in these cases.

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377 **CONCLUSION**

378 Actinomyces associated breast infections are problematic, difficult to diagnose
379 and difficult to treat. They are increasingly recognized in clinical practice, most
380 likely due to a combination of increased awareness and improved diagnostic
381 methods. Further studies are required to clarify the pathogenic role of
382 *Actinomyces* species in various inflammatory conditions which involve the
383 breast, such as periductal mastitis, hidradenitis suppurativa and
384 granulomatous mastitis. These conditions all present with clinical features
385 similar to those seen in Actinomyces breast infections. Taking into account
386 the fastidious nature of *Actinomyces* species, it is quite possible that
387 anaerobic actinomycetes are present significantly more frequently than they
388 are found in these conditions. In particular, it is important to clarify whether
389 longer initial courses of effective antibiotic treatment may prevent recurrences
390 and radical surgery when *Actinomyces* species are isolated in association
391 with these conditions.

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Table 1 Cases of Actinomyces species isolated from breast infections at the Edinburgh Breast Unit over an 8 year period from 2005-2013

Patient	Year	Age (years) / sex	Risk factor(s) / PMH	Type of breast infection	MALDI-TOF identification (p=score)	Molecular identification (16S sequencing)	Co-infecting organisms	No. of infections	Previous potential Actinomyces isolate not identified as such by laboratory	Comments on outcome (incl antibiotic treatment, surgery, resolution)
1	2013	36 F	Nil	Left breast abscess	<i>Actinomyces radingae</i> (p=2.0234) <i>Actinomyces europaeus</i> (p=1.972)	<i>Actinomyces radingae</i> and <i>Actinomyces europaeus</i>	Yes (<i>Peptoniphilus</i> sp.)	1	No	Good response to drainage and clindamycin.
2	2013	52 F	Diabetes	Right breast infra-mammary fold abscess	<i>Actinomyces europaeus</i> (p=1.779)	<i>Actinomyces europaeus</i>	No	1	No	Abscess aspirated and patient treated with flucloxacillin. Complete resolution 3 months later.
3	2013	36 F	Nil	Left breast abscess	<i>Actinomyces odontolyticus</i> (p=2.006)	<i>Actinomyces odontolyticus</i>	No	2	First isolate initially reported as "Diphtheroid" of doubtful significance.	Chronic breast lump slowly increasing in size over 8 months, initially investigated as potential malignancy. Core biopsy revealed changes consistent with chronic abscess and purulent aspirate grew <i>Actinomyces odontolyticus</i> .
4	2013	26 F	Nil	Left breast abscess (infected epidermoid cyst)	<i>Actinomyces neuui</i> (p=2.3314)	<i>Actinomyces neuui</i>	No	1	No	Initial partial response to amoxicillin-clavulanic acid which was changed to ciprofloxacin due to intolerance. Apparent relapse which settled after 6 weeks of amoxicillin. Residual mass excised, pathology showed epidermoid cyst.
5	2013	41 F	Nil	Left breast abscess	<i>Actinomyces radingae</i> (p=2.0348)	<i>Actinomyces radingae</i>	Yes (multiple anaerobe species)	7 (over 10 years)	Yes (sample from 2012 with "diphtheroids" and anaerobes).	Multiple recurrences of breast abscesses, with no growth on culture as patient was already on antibiotics. Sample from 2012 isolated "diphtheroids" and anaerobes. Patient's GP was advised to refer patient for aspiration before starting antibiotics if abscess recurred. This resulted in growth of <i>Actinomyces radingae</i> along with multiple anaerobe spp. Treated with drainage and amoxicillin-clavulanic acid and metronidazole for 2 weeks followed by 3 months of amoxicillin. No further recurrences almost 2 years later.
6	2013	19 F	Smoker / Hidradenitis suppurativa	Breast abscess	<i>Actinomyces</i> species (p=2.0186)	<i>Actinomyces</i> species (closest sequence <i>Actinomyces europaeus</i>)	No	1	No	Breast abscess drained. Patient treated with amoxicillin-clavulanic acid for 1 week with apparent resolution.

Table 1 Cases of Actinomyces species isolated from breast infections at the Edinburgh Breast Unit over an 8 year period from 2005-2013

Patient	Year	Age (years) / sex	Risk factor(s) / PMH	Type of breast infection	MALDI-TOF identification (p=score)	Molecular identification (16S sequencing)	Co-infecting organisms	No. of infections	Previous potential Actinomyces isolate not identified as such by laboratory	Comments on outcome (incl antibiotic treatment, surgery, resolution)
7	2012	34 F	Smoker	Left and right breast abscesses	<i>Actinomyces radingae</i> (p=1.829)	<i>Actinomyces radingae</i>	Yes (<i>Actinobaculum schaalii</i> and multiple anaerobe species)	8	No	Multiple recurrences of breast abscesses over a period of 12 months treated with aspirations and short courses of antibiotics (mostly combinations of flucloxacillin, amoxicillin-clavulanic acid and metronidazole). Referred for mammary fistula and total duct excision of right breast.
8	2012	46 F	Diabetes / Smoker / Hidradenitis suppurativa	Breast abscess	<i>Actinomyces neuii</i> (p=2.076)	<i>Actinomyces neuii</i>	No	2	Yes (sample from 2011 with "diphtheroids" and anaerobes).	Abscess drained and patient treated with antibiotics. Previous episode in 2011 treated with amoxicillin-clavulanic acid and metronidazole.
9	2012	38 F	Smoker	Breast abscess and periductal mastitis	<i>Actinobaculum schaalii</i> (p=not available)	<i>Actinobaculum schaalii</i>	Yes (<i>Streptococcus constellatus</i>)	4 (over 3 years)	Yes (sample from 2011 with "diphtheroids" and alpha-haem streptococci).	Recurrent left breast periductal mastitis and abscess, 4 episodes over 3 years. Treated with antibiotics and sometimes aspiration.
10	2011 - 2012	36 F	Diabetes / Smoker / Hidradenitis suppurativa	Left and right breast abscesses	<i>Actinomyces neuii</i> (p=1.921) and <i>Actinomyces europaeus</i> (p=<2.0)	<i>Actinomyces neuii</i> and <i>Actinomyces europaeus</i>	Yes (Anaerobes)	2	No	Two separate breast abscesses left and right breast 3 months apart. First episode treated with multiple 7 day courses of flucloxacillin and/or amoxicillin. Second episode treated with 3 months of amoxicillin. No recurrence 18 months later.
11	2005	43 F	Smoker	Left periductal mastitis	Not available. Sent to reference laboratory.	<i>Actinomyces europaeus</i>	Yes (Anaerobes)	2	Isolate identified as " <i>Corynebacterium</i> species" and sent to reference laboratory.	Two episodes of periductal mastitis in 1997-98, requiring aspiration (no microbiological data available) and 4 weeks of antibiotics. Presented again in 2005 with an abscess requiring drainage twice 4 weeks apart. Treated with 10 days of oral amoxicillin-clavulanic acid.
12	2005	59 F	Diabetes	Left breast abscess	Not available. Sent to reference laboratory.	<i>Actinomyces europaeus</i>	No	Unknown	Isolate identified as " <i>Corynebacterium</i> species" and sent to reference laboratory.	Information not available.

Table 2. Actinomyces species isolated from breast infections referred to
UK Anaerobic Reference Unit from UK hospitals 1988-2014

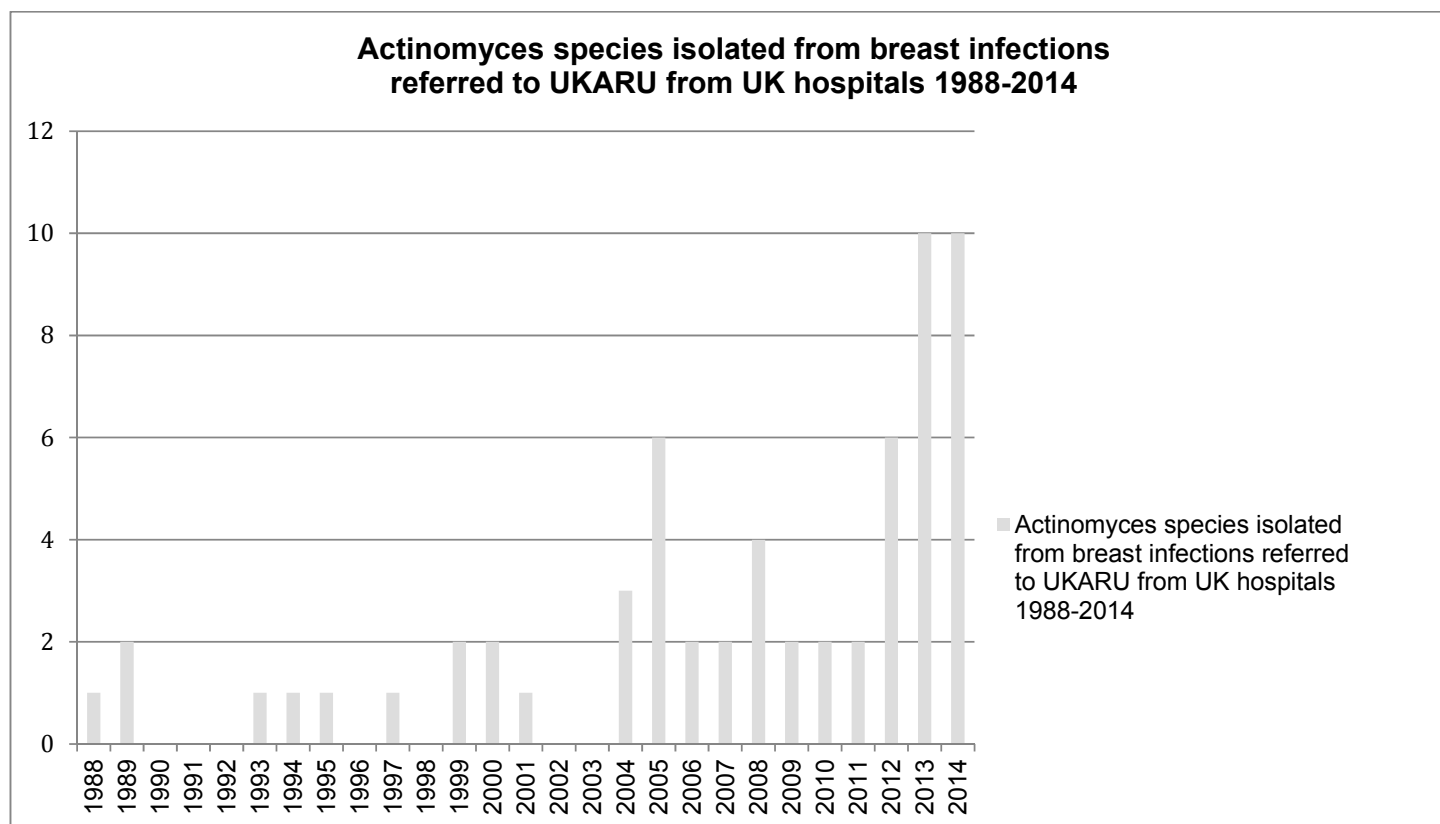


Table 3 - Published cases of *Actinomyces* species isolated from breast infections reported with clinical details since 1994

Patient	Country	Year	Age	Risk factor(s) / PMH	Clinical description	Organism	Method of identification	Co-infecting organisms	No. of infections	Comments on outcome (incl antibiotic treatment, surgery, resolution)	Ref
1	USA	1987	29	Severe periodental disease	Hard 5x4 cm left breast mass	<i>Actinomyces meyerii</i>	Not specified	Diverse anaerobes	1-3 episodes every year for 23 years	Surgical debridement with ampicillin, doxycycline for 4 months	14
2	USA	1987	36	Recurrent periodental abscesses	Hard 4x4 cm right breast mass	<i>Actinomyces meyerii</i>	Not specified	Diverse anaerobes	5 recurrences over 3 years	Surgical debridement with tetracycline, doxycycline for 4 months	14
3	Brazil	2000	66	Diabetes	5 year history of a mass in the left breast. Nipple discharge, cutaneous fistulas.	<i>Actinomyces</i> species	Histopathologic examination. Culture of abscess 4 years previously isolated <i>Actinomyces</i> sp.	None mentioned	2	Abscesses and fibrous tissue drained and resected. Responded to 2 months of IV penicillin followed by oral amoxicillin for 6 months.	15
4	Italy	2005	27	Nil	Unilateral right mastitis, palpable 5 cm nodular lump just beside the right areola.	<i>Actinomyces viscosus</i>	Culture positive. Biochemical tests.	None mentioned	1	One week of an unspecified antibiotic with no response. Then oral amoxicillin/clavulanic acid followed by surgical drainage and excision of the lesion with no further antibiotics. Resolution after 6 year follow up.	16
5	UK	2007	33	Bilateral nipple piercings removed 6 months prior to presentation. On 5mg prednisolone for ulcerative colitis, smoker.	3 week history of right breast pain, swelling, and offensive nipple discharge.	<i>Actinomyces turicensis</i>	Culture positive. Confirmed with 16S rDNA restriction analysis.	Mixed anaerobes	3	Aspiration and amoxicillin/clavulanic acid for 7 days. Worsening symptoms and three more attempts to aspirate over the following 2-week period. Incision and drainage with full excision of abscess wall, followed by ceftriaxone and oral metronidazole for 3 weeks. Complete resolution at follow up 8 weeks later.	17

Table 3 - Published cases of Actinomyces species isolated from breast infections reported with clinical details since 1994

Patient	Country	Year	Age	Risk factor(s) / PMH	Clinical description	Organism	Method of identification	Co-infecting organisms	No. of infections	Comments on outcome (incl antibiotic treatment, surgery, resolution)	Ref
6	UK	2007	38 (male)	Ex-smoker	Right axillary and left subareolar abscess with nipple discharge for 7 months. Left axillary abscess drained 18 months previously.	<i>Actinomyces radingae</i> (isolated from subareolar abscess)	Culture positive. Confirmed with 16S rDNA restriction analysis.	Heavy growth of anaerobes (from right axillary abscess)	3	Incision and drainage of abscesses. Oral amoxicillin and fusidic acid for 6 weeks. Recurrence of <i>A. radingae</i> followed by a prolonged course of oral ciprofloxacin and rifampicin with eventual resolution.	17
7	France	2009	48	Pregnant	Inflammatory breast mass 15 mm. Clinically and radiologically interpreted as carcinoma.	<i>Actinomyces neuii</i>	FNA showed granulomas. Culture positive. Confirmed with 'genetic amplification'.	None mentioned	1	Treated with 3 weeks of oral amoxicillin with resolution	18
8	Iran	2009	30	Nil	Few days history of sudden painful and swollen left breast with multiple fistula formation.	<i>Actinomyces israelii</i>	Morphology on culture	None mentioned	1	Responded to treatment with oral erythromycin for 6 months.	19
9	UK	2010	35	7 months post-partum	3 month history of tenderness and induration in the right breast	Not isolated on culture	Histopathologic examination	Staphylococcus aureus on skin swab	1	Treated with oral penicillin with little improvement. Further treatment with imipenem, coamoxiclav and metronidazole had little effect. Finally treated for over 12 months with oral clindamycin with resolution.	20
10	Iran	2010	48	Psychiatric problem	2 month history of non-tender mass in the left breast.	Not isolated on culture	Histopathologic examination	None mentioned	1	Treated with 4 weeks of intravenous penicillin, followed by oral amoxicillin for 4 months. Fully resolved at 2 year follow up.	21

Table 3 - Published cases of Actinomyces species isolated from breast infections reported with clinical details since 1994

Patient	Country	Year	Age	Risk factor(s) / PMH	Clinical description	Organism	Method of identification	Co-infecting organisms	No. of infections	Comments on outcome (incl antibiotic treatment, surgery, resolution)	Ref
11	France	2010	46	Nil	Recurrent abscess with fistulas	<i>Actinomyces neuii</i>	Not specified	None mentioned	Recurrent abscesses	No improvement with antibiotics. Tumorectomy of the breast. No recurrence after 6 months.	22
12	Spain	2010	48	Nil	Painful erythematous fluctating left breast lump	<i>Actinomyces neuii</i>	Culture positive. Confirmed with 16S rRNA sequencing	None mentioned	1	Resolved with surgical debridement and a course of oral penicillin V	23
13	Switzerland	2011	67	Aortic valve replacement 2 months previous	3 day history of painful 12 cm swelling and hyperaemia of the left breast	<i>Actinomyces europaeus</i>	Culture positive. Confirmed with 16s rRNA and 16s rDNA sequencing	Mixed anaerobic flora	1	3 weeks of oral amoxicillin-clavulanic acid followed by 15 months amoxicillin. At 3 months 3 sinuses formed. Resolved at follow up 6 months later with scar formation.	24
14	India	2012	50	Nil	6 month history of intermittently discharging 6x4 cm right breast mass	<i>Actinomyces israelii</i>	Sulphur granules. Morphology on culture.	None mentioned	1	No information	25
15	India	2012	61	Diabetes	6 month history of 5 x 6 cm mass in left breast	Not isolated on culture	Histopathologic examination	None mentioned	1	Treated with unspecified antibiotics. Doing well on follow up.	26
16	India	2012	32	Nil	3 week history of 7 x 8 cm right breast mass	Not isolated on culture	Histopathologic examination	None mentioned	1	Resolved after a course of unspecified antibiotics.	27
17	USA	2013	40	Nil	2 week history of 3 cm tender right breast mass with overlying erythema	<i>Actinomyces odontolyticus</i>	Not specified	None mentioned	1	Cefalexin for 1 week with resolution after 2 weeks of oral penicillin V.	28

Table 4. Combined number and species of *Actinomyces* breast infections from tables 1-3 and from reference 7.

	Lothian cases*	Anaerobe Ref Unit cases†	Published cases‡	Total
<i>A. europaeus</i>	5	12	1	18
<i>A. funkeii</i>	0	2	0	2
<i>A. israelii</i>	0	0	4	4
<i>A. massiliense</i>	0	1	0	1
<i>A. meyerii</i>	0	0	4	4
<i>A. neuui</i>	3	13	3	19
<i>A. odontolyticus</i>	1	5	4	10
<i>A. radingae</i>	3	11	1	15
<i>A. turicensis</i>	0	15	1	16
<i>A. urogenitalis</i>	0	2	0	2
<i>A. viscosus</i>	0	0	1	1
<i>A. species</i>	1	0	4	5
<i>Actinobaculum schaalii</i>	1	0	0	1
Not cultured	0	0	4	4
Total	14	61	27	102
	* Two Lothian cases had two different <i>Actinomyces</i> sp. isolated	† Isolates referred from Lothian removed from this column to avoid double counting	‡ Includes 10 cases from ref 7 with no clinical details	